Phospholipid Biosynthesis in Mature Human Erythrocytes

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Abstract. Mature human erythrocytes were tested for their ability to synthetize membrane phospholipids from simple precursors: [^{32}P]-orthophosphate ($^{32}P_i$), [U- ^{14}C] glycerol, [U-^{14}C] glucose, [U-^{14}C] serine, and [U-^{14}C] choline. The incorporation of these labels into phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS), phosphatidylinositol (PI), phosphatidic acid (PA), lysophosphatidylcholine (lyso-PC), phosphatidylinositol-4-phosphate (PIP), and phosphatidylinositol-4,5-bisphosphate (PIP_2) was measured. All the phospholipids tested incorporated $^{32}P_i$, glycerol, and glucose in a time dependent manner. According to the rate of $^{32}P_i$ incorporation, three groups of phospholipids could be distinguished: 1) PA, PIP_2, PIP, lyso-PC; 2) PI and PS; 3) PC and PE, which incorporated 5×10^3, 40, and 6 nmol $^{32}P_i$/mmol phospholipid per 1 h, respectively. Moreover, [U-^{14}C] serine and [U^{14}C] choline were found to incorporate into phospholipids, and PS-decarboxylase activity could be measured. The possibility that the observed incorporation was due to contamination with bacteria or other blood cells could be ruled out. Our results bring evidence for de novo phospholipid synthesis of human red blood cells.

Key words: [^{32}P] orthophosphate incorporation — Phosphatidylserine decarboxylase — Phosphoinositides — Human erythrocytes

Introduction

It is generally accepted now that mammalian erythrocytes have a very limited ability to synthetize phospholipids de novo from simple precursors such as inorganic phosphate, glucose, and glycerol. The enzymes responsible for the turnover of phosphodiester groups have been shown to be absent in red blood cells (van Deenen and de Gier 1974) and there is no evidence for the turnover of the glycerol backbone of the major erythrocyte phospholipids (Allan 1982). For phospholipid renewal only exchange of complete phospholipid molecules between serum and erythrocyte membrane has been supposed (Shohet 1970;

Renooij et al. 1976). The majority of studies on $^{32}P_i$ incorporation have shown most of the label in PA, PIP_2, and PIP only. Reed (1968) concluded that PA is the only lipid which in rat erythrocytes is clearly labelled with $^{32}P_i$, while Schneider and Kirschner (1970), when studying swine erythrocytes, found ^{32}P exclusively in the fractions PIP_2 and PIP. In a study by Koutouzov et al. (1982) rat erythrocytes were incubated for 90 min and the ^{32}P label was incorporated mostly into PIP_2 and PIP with less than 1 % of total radioactivity in PA. Allan et al. (1976) found after 20 h incubation of human erythrocytes with $^{32}P_i$ radioactivity to be present only in PA and polyphosphoinositides in a ratio of 9 : 1. PA, PIP, and PIP_2 were the only phospholipids labelled in rabbit ghosts incubated in the presence of Mg-[^{32}P] ATP for 80 min (Quist 1982).

Thus only monoester phosphates of PA, PIP_2, and PIP seem to have a measurable turnover in red blood cell membrane.

In studying the time course of $^{32}P_i$ incorporation into polyphosphoinositides and phosphatidate in human erythrocytes we found that they are not only phospholipids labelled during 1—5 h incubation. We could show that the incorporation of metabolic precursors into red blood cell phospholipids is not entirely absent as claimed previously.

Materials and Methods

Materials

[^{32}P] orthophosphate (carrier free), [U-^{14}C] glycerol (S. A. 262 MBq/mmol), [U^{14}C] glucose (S. A. 3 710 MBq/mmol), [U-^{14}C] serine (S. A. 3 890 MBq/mmol), and [U-^{14}C] choline (S. A. 548 MBq/mmol) were obtained from ÚVVVR (Czechoslovakia), Ficoll was from Pharmacia (Sweden), Ampiciline from Spofa (Czechoslovakia), and the equipment for TLC form Merck (FRG). The organic solvents used were glass redistilled. All other chemicals were of highest purity available.

Preparation of erythrocytes

Blood was drawn from healthy male volunteers into acid/citrate/dextrose solution and kept at 4 °C. The material was used at the same day. Following centrifugation at 600 x*g* for 30 min the platelet rich plasma was discarded. After a subsequent centrifugation (1500 *g*, 10 min) the buffy coat was sucked off and the erythrocyte pellet washed with 2 vol. of 154 mmol.l^{-1} NaCl, 1.5 mmol.l^{-1} Hepes (pH 7.2). Contaminating cells were eliminated using differential centrifugation through Ficoll (11.7 % wůw) — Hypaque (8.05 % w/w) solution (density 1090 kg.m^{-3}). The erythrocyte suspension

Abbreviations used: PA — phosphatidic acid, PI — phosphatidylinositol, PS — phosphatidylserine, PC — phosphatidylcholine, PE — phosphatidylethanolamine, lysoPC — lysophosphatidylcholine, PIP_2 — phosphatidylinositol-4,5-bisphosphate, PIP — phosphatidylinositol-4-phosphate, TLC — thin layer chromatography, S. A. — specific activity, Hepes-4-(2-hydroxyethyl)-1-piperazineethanesulphonic accid, Tris-2-amino-2-hydroxymethylpropane-1,3-diol.

was layered on the same volume of Ficoll-Hypaque and centrifuged at 3500 x*g* for 60 min at 18 °C (on a VAC ultracentrifuge Janetzki). Red blood cells passed through the Ficoll solution leaving the white cells at the interface. For further use, only the lower half of the Ficoll layer was collected. This procedure was repeated twice and resulted in erythrocytes essentially free of other blood cells. The final sediment was washed three time with 3 vols. of ice-cold NaCl-Hepes buffer. All the procedures were performed with sterile glassware and solutions to minimize possible microbial contamination.

Incubation conditions

The incubations were carried out under sterile conditions. In some experiments parallel incubations with 50 $mg.l^{-1}$ of Ampicilin were performed to avoid bacterial contamination. The incubation vessels usually contained 1 ml of packed erythrocytes in 2 ml of the final volume and the radioactive precursors adjusted to isotonicity. The samples were incubated in a stirred water bath at 37 °C for periods as indicated.

Isotope doses were 29.6 MBq of $^{32}P_i$, 2.96 Mbq of [U-^{14}C] glucose, 1.11 MBq of [U-^{14}C] glycerol, 1.85 MBq of [U-^{14}C] serine and 1.85 MBq of [U-^{14}C] choline. The low calcium medium (M1) contained (final concentrations in $mmol.l^{-1}$): Hepes 40, adenosine 1, inosine 0.5, glucose 8, Na_2HPO_4 0.2, KCl 31.2, K_2EDTA 10, NaCl 65.0, free Mg^{2+} 0.15, and free Ca^{2+} 0.04 $\mu mol.l^{-1}$. The high calcium medium (M2) was indentical with M1 except that the concentration of NaCl was 62.0 $mmol.l^{-1}$ and that of free Ca^{2+} 1.5 $mmol.l^{-1}$. At the end of the incubation 5 vols. of ice-cold NaCl-Hepes buffer were added, the erythrocytes were quickly pelleted and washed once more in ice-cold NaCl-Hepes buffer.

Ghost preparation

In samples to be analysed for polyphosphoinositides erythrocyte membranes were isolated prior to the extraction. The washed erythrocyte pellet was lysed in 15 vols. of ice-cold 183 $mmol.l^{-1}$ imidazole buffer (pH 7.4) and sedimented by centrifugation at 15,000x*g* for 10 min at 4 °C. The supernatant was discarded and the procedure repeated two times.

Phospholipid extraction and separation

Intact erythrocytes were suspended in 6 vols. of methanol and the mixture was adjusted with chloroform to give a chloroform/methanol ratio of 1 : 1. After 10 min of extraction the mixture was centrifuged (1500 × *g*, 10 min), the supernatnt collected and the sediment reextracted with 6 vols. of chloroform/methanol (2 : 1).

The combined extract was washed with 0.2 vol. of 0.5 $mol.l^{-1}$ KCl. The chloroform phases were evaporated in vacuum and applied to TLC plates. The neutral extracts were processed on two-dimensional TLC as described by Rouser et al. (1969) using chloroform / methanol / concentrated ammmonia / water (70 : 25 : 4 : 1) and chloroform / methanol / acetone / acetic acid / water (140 : 25 : 35 : 20 : 9) in the first and second direction, respectively.

Polyphosphoinositides were extracted by acidic extraction. Erythrocyte membrane suspension was mixed with 3 vols. of chloroform /methanol /concentrated HCl (30:60:0.5) until the membranes were completely dissolved. The extraction was carried out at 4 °C. The system was partitioned into two phases by the addition of 0.2 vol. of 0.1 $mol.l^{-1}$ HCl. The interphasic material was extracted with the original amount of chloroform / methanol / concentrated HCl mixture and 0.2 vol. of 0.1 $mol.^{-1}$ HCl were added. The upper phases from the first and second extraction were collected and reextracted. The lower phases from all the three extractions were collected and washed with the same volume of methanol / 0.5 $mol.l^{-1}$ KCl (2 : 3) mixture. The upper phase was washed

once more with 0.8 vol. of chloroform. The lower phases were combined and evaporated in vacuum. Polyphosphoinositides were separated on silica gel precoated plates (Merck, FRG) impregnated with 1 % oxalate. The solvent system used was chloroform / methanol / acetone / acetic acid / water (40 : 15 : 13 : 7) as described by (Shaikh and Palmer 1977).

Phospholipids were detected by iodine vapours and by autoradiography. The spots were scrapped off and radioactivity was detemined in a toluene scintillation cocktail on a Beckman scintillation counter. The quenching was compensated for automatically. The indentity and the purity of phospholipids was checked after hydrolysis according to Clarke and Dawson (1981). Phosphate was estimated according to Rouser et al. (1969). With phosphate contents lower than 0.01 μmol per sample the modified method of Duck-Chong (1979) was used.

Phosphatidylserine decarboxylase assay

Radioactive phosphatidyl [U-^{14}C] serine was prepared as described by Butler and Morell (1983). Erythrocyte membranes were prepared by lysing washed erythrocytes in 15 vols. of 30 mmol.l^{-1} Tris / maleate buffer at 4 °C. After 30 min the suspension was centrifuged at 100,000 xg for 60 min (VAC ultracentrifuge, Janetzki), resuspended in 50 mmol.l^{-1} Tris / maleate buffer and used immediately.

The enzyme assay was performed according to Suda and Matsuda (1974). The incubations were done in Warburg flasks at 37 °C for 3 hours in a final volume of 1.5 ml. The main compartment contained erythrocyte membrane preparation (15 mg of protein) and phosphatidyl [U-^{14}C] serine emulsion (300,000 dpm) in 50 mmol.l^{-1} Tris / maleate buffer (pH 6.0). For the measurement of radioactive CO_2 the center well of the flasks contained 0.2 ml of methylbenzethonium hydroxide soaked in a piece of filter paper, and the side arm contained 0.5 ml of 3 mol.l^{-1} $HClO_4$ (to liberate CO_2 formed after incubation). The content of the center well was transferred quantitatively into 10 ml of toluene scintillation cocktail. When measuring radioactive PE the reaction was stopped by adding 6 ml of chloroform / methanol (1 : 2). The mixture was then processed as described above.

Others

To test the possibility of nonenzymatic $^{32}P_i$ incorporation and/or adsorption phospholipids from unlabelled erythrocytes were purified on TLC and sonicated in the incubation medium in the presence of Ampicilin. The emulsion was then incubated with $^{32}P_i$ for 5 h and analyzed for phospholipids.

In some experiments ^{32}P labelled PA, PIP, and PIP_2 were added to unlabelled phospholipid extracts to rule out the possible incomplete separation of lipids.

To check the contamination of erythrocytes with other blood cells smears were made and stained with methylene blue or by the Pappenheim method (May-Grünwald and Giemsa-Romanowski reagents). The reticulocytes were stained according to Kolmer and Boerner (1945).

The protein content of erythrocyte membranes was estimated with the biuret method after prior solubilization with deoxycholate (Gornall et al. 1949). Corrections were made to heme color of the samples.

Results

$^{32}P_i$ *incorporation*

The time course of $^{32}P_i$ incroporation into phospholipids during incubation of human erythrocytes in medium M2 is shown in Fig. 1. The incorporation

increased throughout the period of observation without changes in relative proportions of the individual phospholipid contents. The Figure shows that $^{32}P_i$ is incorporated into red blood cell phospholipids at different rates. The highest incorporation rate was found for PA, PIP_2, lyso-PC, and PIP (5×10^3 nmol $^{32}P_i$ mmol phospholipid/1 h for PA and 40 nmol $^{32}P_i$/mmol phospholipid/1 h for both PI and PS. The third group of phospholipids, PE and PC (57.8 % of the total phospholipid content in red blood cell) show low but still significant $^{32}P_i$ incorporation (in the order of 6 nmol $^{32}P_i$/mmol phospholipid/1 h for PC). Incorporation into sphingomyelin was negligible.

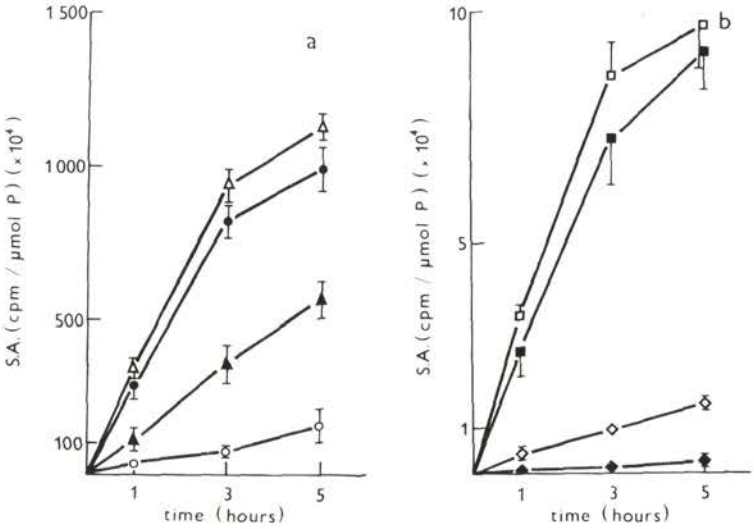

Fig. 1. Time course of $^{32}P_i$ incorporation into phospholipids of human erythrocytes. The erythrocytes were incubated in medium (M2) and processed as described in Methods.
a: ● PA, △ PIP_2, ○ lyso PC, ▲ PIP; *b*: □ PI, ■ PS, ◇ PC and ◆ PE. Vertical bars indicate S. E. M., $n = 6$.

[*U*-^{14}*C*] *glucose incorporation*

The specific activities of phospholipids after 1, 3, and 5 h of incubation in medium M2 in the presence of [U-^{14}C] glucose are summarized in Table 1. The highest S. A. were measured in PA and lysoPC, followed by PI, PS, PC, and PE in decreasing order. With labelled glucose, S. A. of PI was 10 times higher than that of PS. As with $^{32}P_i$, [U-^{14}C] glucose incorporation increased continuously throughout the 5 h period.

[U-^{14}C] glycerol incorporation

The time course of [U-^{14}C] glycerol incorporation into phospholipids of human erythrocytes after 0.5, 1, 3, and 5 h of incubation is shown in Fig. 2. The experiment suggests both synthetic and degradation metabolism of at least those phospholipids that have high turnover rates. We suppose that the externally added glycerol was quickly metabolized and S. A. of its pool in the cell declined shortly afterwards. This was due to glycerol produced form unlabelled glucose. As shown in Fig. 2*a* the highest S. A. was found for PI at 0.5 h of incubation. The increase-decrease pattern of incorporation was found in PA, PI, and partly in lysoPC. In contrast a continuous increase in S. A. was observed for PC and PE.

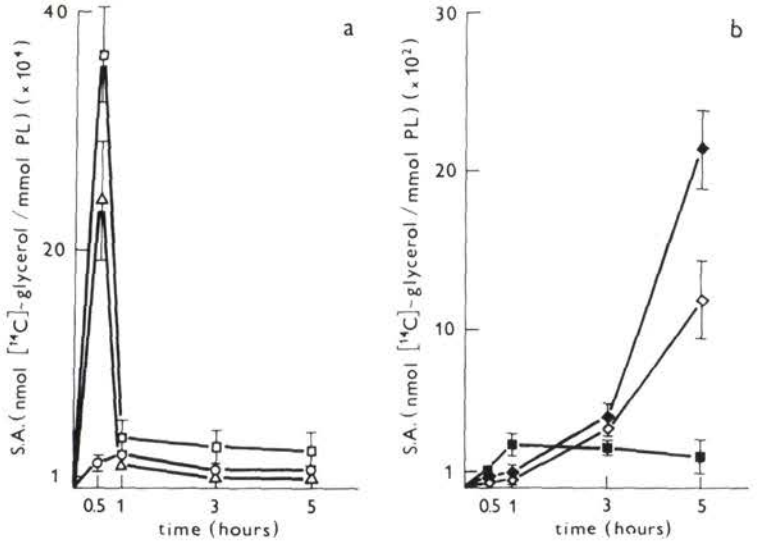

Fig. 2. Time course of [U-^{14}C] glycerol incorporation into phospholipids of human erythrocytes. The erythrocytes were incubated in medium (M2) and processed as described in Methods. *a*: □ PI, ○ lyso PC, △ PA; *b*: ◆ PE, ◇ PC, and ■ PS. Vertical bars indicate S. E. M., $n = 6$.

[U-^{14}C] serine and [U-^{14}C] choline incorporation

[U-^{14}C] serine incorporation into erythrocyte membrane phospholipids was assessed after 5 h incubation in media M1 and M2. The incorporation rates of PS, PE, and lyso-PC, (Table 2) are very similar contrast with the low S. A. of PC.

The incorporation of (U-^{14}C] choline after 5 h of incubation was not dependent of extracellular calcium concentration (Table 2). However the labell-

Table 1. Time course of [U-^{14}C] glucose incorporation into phospholipids of human erythrocytes. The erythrocytes were incubated in medium M2 and neutral extraction of phospholipids was performed. Results are means ± S. E. M., $n = 6$.

Phospho-lipids	S. A. (nmol [U-^{14}C] glucose/mol phospholipid)		
	Incubation time (h)		
	1	3	5
PA	2 132 000 ± 547 000	7 540 000 ± 1 285 000	44 826 000 ± 8 044 000
1PC	225 000 ± 52 300	277 000 ± 54 400	2 562 000 ± 627 000
PI	118 000 ± 21 500	161 000 ± 38 500	308 000 ± 65 000
PS	17 300 ± 1 050	44 600 ± 16 000	263 000 ± 62 000
PC	6 800 ± 779	15 900 ± 1 680	24 700 ± 1 720
PE	2 300 ± 398	7 200 ± 1 630	13 300 ± 1 370

Table 2. Incorporation of [U-^{14}C] serine and [U-^{14}C] choline into phospholipids of human erythrocytes. The erythrocytes were incubated in media M1 and M2 for 5 h at extracellular Ca^{2+} concentrations of 0.04 μmol . l^{-1} and 2 mmol . l^{-1}, respectively. Neutral extraction of phospholipids was carried out as described in Methods. Results are means ± S.E.M., $n = 4$.

Phospholipid	S.A. (nmol U-^{14}C serine/mol phospholipid)	
	M1 (0.04 μmol . l^{-1} Ca^{2+}	M2 (2 mmol . l^{-1} Ca^{2+})
PS	1 380 000 ± 135 000[a]	5 924 000 ± 702 000[a]
PE	1 429 000 ± 128 000	859 000 ± 432 000
PC	54 600 ± 2 610[a]	30 200 ± 1 750[a]
1PC	1 386 000 ± 315 000[a]	4 136 000 ± 851 000[a]
	S. A. (nmol U- ^{14}C choline/mol phospholipid)	
	M1 (0.04 μmol.l^{-1} Ca^{2+})	M2 (2 mmol.l^{-1} Ca^{2+})
PC	97 506 ± 5 880	108 137 ± 19 524

[a] Statisticaly significance at $P < 0.05$

ing pattern of [U-^{14}C] serine incorporation differed markedly with different extracellular Ca^{2+} concentrations.

Phosphatidylserine decarboxylase assay

Three types of control incubations were done to exclude possible artifacts: 1) [U-^{14}C] serine was added instead of labelled PS, 2) before the addition of PS emulsion the membranes were boiled for 10 min to destroy the enzyme activity, and 3) PS decarboxylase inhibitor was added (hydroxylamine to a final concentration of 5 mmol.l^{-1}).

In a typical experiment, 1000—2000 dpm were recorded per sample in $^{14}CO_2$ or phosphatidyl [U-^{14}C] — ethanolamine (EKG = 90 dpm). This value represented 0.8—2.0 % of the originally present PS, and 160—251 % of controls 2) and 3). The contribution of (U-^{14}C] serine decarboxylation was 10 times lower than the rate of nonenzymatic PS decarboxylation. The PS decarboxylase activity in our preparation was 2,4—6.0 nmol PS decarboxylated/100 mg protein per 1 h ($n = 3$). Although very small, this value is still significantly higher than nonenzymatic PS decarboxylation.

Contribution of the contaminating cells and other factors

In our experimental protocol special attention was paid to the purity of the red blood cells used. Most platelets were discarded after the first low speed centrifugation. We tested the platelet population in separate experiments. No thrombocyte adhesion and/or lysis occurred. After a subsequent centrifugation the buffy coat was carefully removed thus substantially reducing white blood cell contamination. The remaining white cells, platelets, and reticulocytes were efficiently removed during the Ficoll-Hypaque density gradient centrifugation (see Methods).

In erythrocytes prepared in this a way, the concentrations of nucleated cells never exceeded 10^4/ml, and reticulocytes concentrations were below 10^5/ml. This is equivalent to 1 nucleated cell per 10^6 of erythrocytes.

The possible contribution of the metabolism of contaminating cells to the observed turnover of phospholipids in erythrocyte preparations is shown in Table 3. It is apparent that even in a concentration of 6×10^5/ml the contaminating cells contribute less than 10 % to the total incorporated radioactivity of the control sample with erythrocytes (with the exception of PE).

To rule out the possibility of bacterial contamination our experiments were performed under sterile conditions (see Methods). In addition, parallel incubations with antibiotic were run. Ampicilin, which inhibits bacterial growth, did not abolish incorporation (data not shown), suggesting that the role of bacterial contamination in the observed incorporation into erythrocyte phospholipids can be neglected.

Special experiments were designed to estimate nonenzymatic $^{32}P_i$ incorporation in and/or adsorption to phospholipids of human erythrocytes. Phospholipids extracted from erythrocytes were purified by TLC, eluted, and sonicated. After 5 hours of incubation in the presence of $^{32}P_i$ only background level of incorporation was found (Table 3). Based on the above data we conclude that the incorporation of precursors into phospholipids of human erythrocytes is due to enzyme activities of the mature human erythrocyte itself.

Table 3. Contributions of white blood cell metabolism and nonenzymatic reactions to $^{32}P_i$ incorporation into phospholipids in mature human erythrocytes. Incubations for 5 hours in the presence of 50 mg/l Ampicilin, the final sample volume was 2 ml. Erythrocytes (control) were suspended at 50 % hematocrite, white blood cells at 6×10^2/ml. Liposomes represents sonicated phospholipids in ammounts equal to the phospholipid content in control erythrocyte sample (see Methods for details). Phospholipids from all samples were extracted and separated by TLC. Data given show activities (cpm) measured in the respecting phospholipids after background substration. Mean ± S. E. M., $n = 6$.

Phospholipid	Activity (cpm)		
	white blood cells 6×10^5/ml	erythrocytes control	liposomes
PA	509 ± 51	106 100 ± 9 880	27 ± 5
PI	67 ± 4	751 ± 14	1
PS	108 ± 3	5 350 ± 453	11 ± 2
lyso PC	—	1 010 ± 247	2
PC	285 ± 19	1 359 ± 97	58 ± 7
PE	137 ± 4	175 ± 17	15 ± 7

Discussion

Our results suggest that human erythrocytes are able to renew, at least part by their phospholipids. As can be seen from Fig. 1, significant incorporation of $^{32}P_i$ into PA, PIP_2, PIP, lysoPC, PI, PS, PC and PE was observed during 1—5 h of incubation of human red blood cells in vitro. The different rates of $^{32}P_i$ incorporation into the above phospholipids probably explain the lack of literary reports of $^{32}P_i$ incorporation into PC and PE. High doses of the isotope (1 MBq/ml) should be used in the incorporation experiments to make such a slow process detectable. There has been only one report that provided evidence for the turnover of the diester phosphate group of PI and PS (Jacob and Kranovski 1967).

Considering the generally accepted assumption that $^{32}P_i$ is incorporated only into erythrocyte PA and polyphosphoinositides, we checked the possible role of contamination. Using sterile buffer together with antibiotic we could rule out effect of microorganisms and their growth during the incubation. In addition, the contribution of metabolically active reticulocytes and white blood cells to the incorporation into erythrocyte phospholipids was estimated to be below 10 %. These measurements were done at concentrations of the contaminating cells 5 times higher than the actual concentrations in our samples. Finally, we could rule out artifacts originating from nonenzymatic reactions.

In and other set of experiments the time course of [U-^{14}C] glucose and [U-^{14}C] glycerol incorporation was studied (Table 1 and Fig. 2). The results suggest that there is a glycerol backbone-turnover of erythrocyte phospholipids.

Evidence for PS decarboxylase (E. C. 4. 1. 65) activity in erythrocytes is lacking. In mammalian tissues this enzyme has been found in liver, where it has been localized in the inner mitochondrial membrane (Van Golde et al. 1974) and in brain (Butler and Morell 1983). Suda and Matsuda (1974) reported 13.8 nmol of PS decarboxylated per mg protein/1 h for mouse liver homogenates. Under similar conditions, we measured a maximum of 0.06 nmol of PS decarboxylated per mg protein/1 h. This enzyme activity is very low, similarly as other activities implicated in the incorporations od the radioactive precursor described above. However, it could not be explained as a nonenzymatic process.

Based on experiments with [U-^{14}C] glucose and [U-^{14}C] glycerol, and on $^{32}P_i$ incorporation we can interprete our findings by suggesting the ability of human erythrocytes to complete by synthetize the glycerol backbone. Once synthetized, the glycerol backbone and diester phosphate can appear in all the phospholipids studied.

The extremely low rates of incorporation of all precursors into PC and PE might explain the lack of evidence for their de novo synthesis in human erythrocytes and the suggestion that another mechanism is necessary in vivo for the renewal of these major erythrocyte phospholipids. However, the physiological significance of a high lyso-PC turnover, observed also by Renooij et al. (1976) remains to be elucidated.

The enzymes, which catalyze the synthesis of PI from CDP-diacylglycerol (and PC form diacylglycerol) or decarboxylation of PS to PE are not bound to the plasma membrane. For example, the synthesis of PI form CDP-diacylglycerol is known to proceed in the microsomal fraction (Strunecká and Zborowski 1975; Williamson and Morre 1976). However recent reports have indicated that the PI may be partly synthetized even in the plasma membrane as suggested in the pituitary by Imai and Gershengorn (1987). Alternatively, phospholipid synthetizing reactions may be part of membrane structures, remained in the erythrocyte. Lew and García-Sancho (1985) have indeed found in erythrocytes vesicles of uknown origin that accumulate Ca^{2+}.

The ability to synthetize PI might help the cell to maintain a high content of polyphosphoinositides throughout its life in the bloodstream.

Thus, even a very low synthetic activity as observed in our experiments, may be of considerable importance for the red blood cell physiology.

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